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EXPERIMENTAL TRANSFORMATIONS OF BIPOLAR FORMS IN *CORYMORPHA PALMA.*

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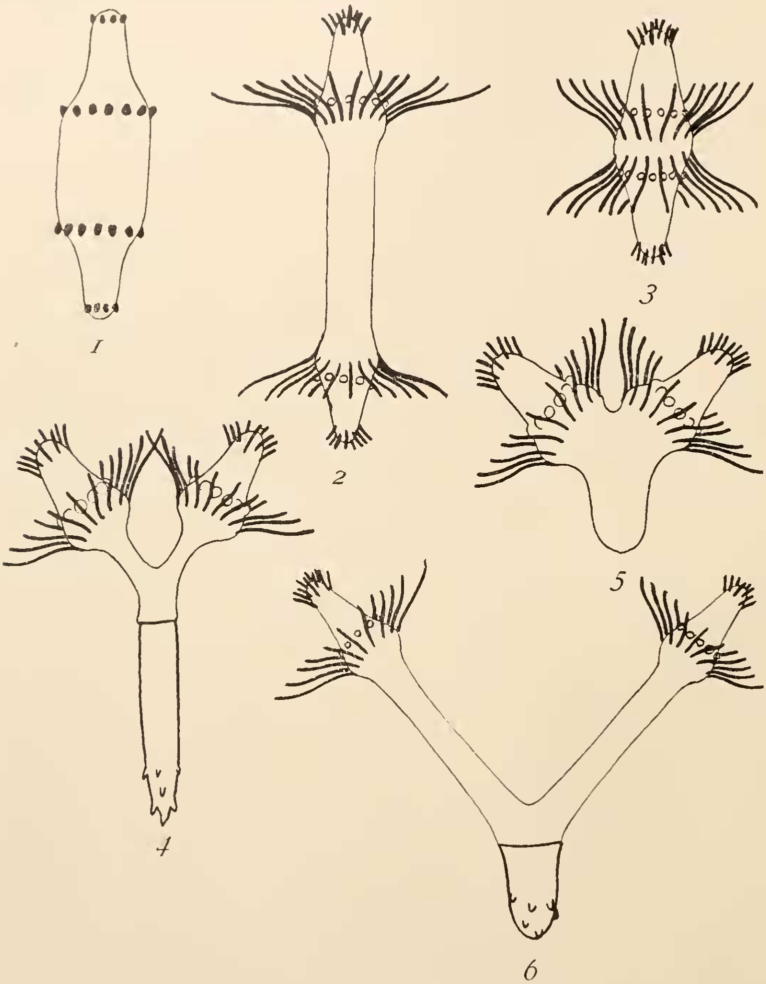
It has been shown in earlier papers that the localization and development of a basal end in the reconstitution of stem pieces of *Corymorpha* may be determined by the dominance of more distal parts (Child, '26*b*, '27*c*), by contact with or nearness to the bottom (Child, '26*b*, '27*a*, *b*) and by the inhibiting action of various agents (Child, '27*a*, *b*, *c*). In normal development the base arises from the low end of the primary gradient (Child, '26*a*), a region of relatively low metabolism, but contact with, or nearness to the bottom and various inhibiting agents, or both acting together, may determine basal ends anywhere, even as mosaic parts on other body regions (Child, '26*b*). Evidently these external factors induce physiological conditions similar to those which arise in embryonic development at the low end of the primary gradient. The present paper gives the results of a few experiments on the transformation of bipolar forms resulting from reconstitution into other forms, its purpose being merely to record the occurrence of such transformations under certain experimental conditions. All figures are drawn from observed and recorded cases. Regions inclosed in perisarc are indicated by heavier line than naked regions and perisarcal accumulations not in direct contact with the body surface are indicated by dotting. Except in case of the very small individuals, the figures are diagrammatic as regards numbers of tentacles.

Transformation of Bipolar into Bipolar-unipolar Forms.

Bipolar forms are those in which a hydranth develops from both distal and proximal end of a stem piece. As shown else-

where (Child, '26*b*), bipolar forms may range from extreme apical ends including merely mouth and hypostome, to complete hydranths with a considerable length of stem between them. Bi-



Figs. 1–6. Bipolar and bipolar-unipolar forms resulting from reconstitution of stem pieces.

polar forms of the sort used in these experiments are shown in Figs. 1–3. Fig. 1 is an earlier, Fig. 2 a later stage of a form with considerable length of stem between the two hydranths, such as ordinarily develops from the longer stem pieces. Fig. 3 shows

a bipolar form from a shorter piece, consisting of hydranths only. Bipolar-unipolar forms (see Child, '26*b*) are bipolar distally and unipolar proximally (Figs. 4–6). The two hydranths develop from the two cut ends of the piece, the unipolar proximal region from the lateral stem region between the hydranths. The proximal region represents a new polarity at right angles to the original.

By means of inhibiting conditions it is possible to transform bipolar forms such as Figs. 1–3 into bipolar-unipolar forms like Figs. 4–6, although the bipolar forms never develop a unipolar proximal region as long as they are kept under good conditions Extensive experimentation along this line has not been undertaken, but several agents have been used, viz., alcohol, two and three per cent., NH_4Cl $m/300$ and ethyl urethane $m/300$ and $m/100$ and in case of one lot of pieces sea water which had stood for some hours in a new galvanized bucket was found very effective as an inhibiting agent. The same transformations appeared in all agents used. The bipolar forms were placed in the agent at earlier (Fig. 1) or later stages (Figs. 2, 3) of development, but only after it was certain that they were developing as symmetrical bipolar forms.

In most cases they remained in the agent one to two days and were then returned to sea water. One series, however, remained in alcohol two per cent. for twelve days, the solution being renewed daily. During exposure to the agent the tentacles are usually reduced to mere stumps or disappear entirely, the distal regions in most cases disintegrating and the stumps undergoing resorption. Hydranths and the stem if it is present, undergo marked reduction in size, particularly in the longer exposure periods and in some cases the manubrium of one or both hydranths may show some disintegration. On return to water the pieces remain undisturbed for two days or more and during this time redevelopment of hydranths and tentacles takes place. If the degree of inhibition is not too extreme the basal region may develop during exposure to the agent, otherwise it develops after return to water.

Fig. 7 shows the transformation of a bipolar form like Fig. 2 after alcohol two per cent. for one day, then alcohol three per cent. for a second day. The figure represents a stage three days after return to water. A proximal stem region and basal end have

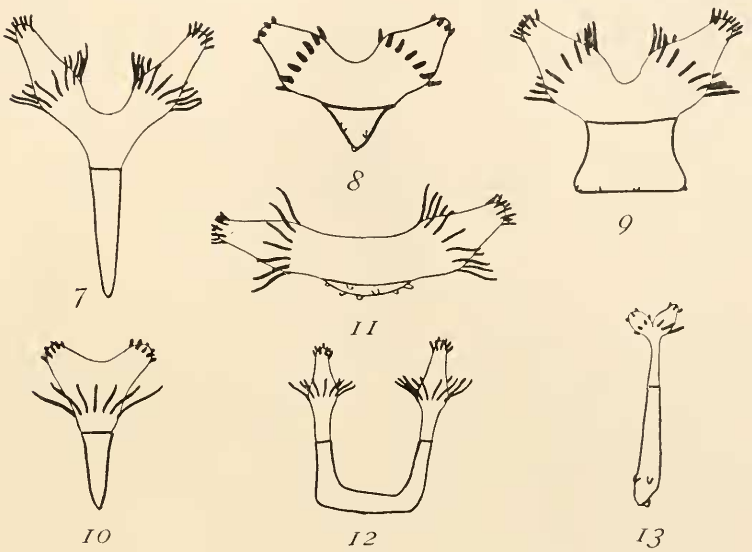
developed from what was originally the lateral stem region between the hydranths. A similar case is shown in Fig. 8. This form, originally bipolar like Fig. 2 but somewhat shorter, was in NH_4CL *m*/300 one day then in sea water two days. At this time redevelopment of tentacles and development of the new base were not far advanced, as Fig. 8 indicates. Fig. 9 was transformed from a piece like Fig. 1 after forty hours in alcohol two per cent. followed by two days in sea water. In this series the pieces were subjected to inhibition at a relatively early stage of the bipolar development and the new basal end was larger in all than when later stages were used.

In cases in which the degree of inhibition is somewhat greater, either because of higher individual susceptibility or because of higher concentration or longer exposure to the agent, modification of form may be more extreme. In the case of Fig. 10, for example the piece was originally bipolar like Fig. 2 but somewhat shorter. It was subjected to alcohol two per cent. for one day, then to alcohol three per cent. for a second day and then remained three days in sea water. As Fig. 10 shows, the proximal regions of the two hydranths have undergone fusion and instead of the two complete sets of proximal tentacles originally present, only one set now appears. In this case there has been not only reorganization of the lateral stem region into a base, but extensive reorganization of the hydranths has also occurred. Other similar cases have been observed.

Figs. 11 and 12 show two stages of the transformation in the toxic sea water. The form was originally bipolar like Fig. 1, but under the inhibiting conditions developed the basal region with holdfast outgrowths within twenty-four hours on the part in contact with the bottom. The basal region continued to grow at the expense of other parts and after four days the condition shown in Fig. 12 was attained. This case was not observed further, but forms of this sort usually separate sooner or later into two individuals, the separation occurring through the gradual atrophy of some portion of the connecting basal region. The region undergoing atrophy apparently becomes a sort of no-man's-land between the two individuals and its cells may be at one time influenced by one, at another time by the other and perhaps some-

times by neither. This absence of definite function probably determines the atrophy.

The case shown in Fig. 13, originally bipolar like Fig. 2 was in alcohol two per cent. for twelve days. During this time it decreased greatly in size, largely because of disappearance of the axial parenchyma, the tentacles disappeared completely and the form of the hydranths approached that of early developmental



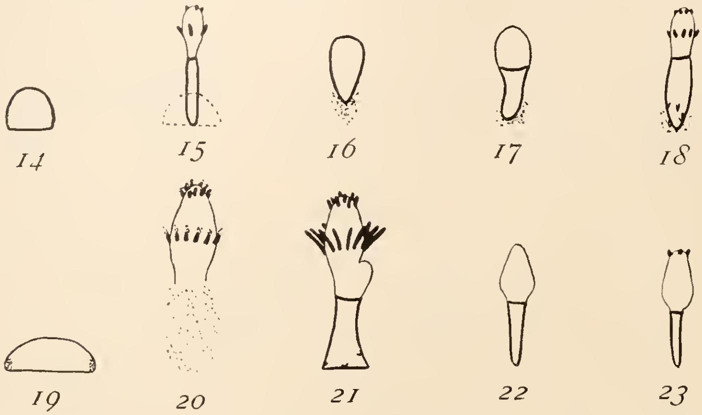
Figs. 7–13. Bipolar-unipolar forms transformed from bipolar forms by inhibiting conditions.

stages. After return to water redevelopment of the two hydranths began and a basal tip developed from the side of the stem in contact, underwent elongation and became disproportionately large at the expense of other parts. Fig. 13 shows the condition seven days after return to water. It will be noted that the hydranth on the left appears to be more inhibited than the other. As will appear below, the two hydranths of a bipolar form often show a considerable difference in susceptibility.

Transformation of Bipolar into Unifolar Forms.

The first experiments on subjection of bipolar forms to inhibiting conditions were performed in 1910 and it was found that some

bipolar forms after several days under inhibiting conditions re-developed as completely unipolar forms. Work had to be discontinued before much experiment along this line was possible, but the observations made on the changes occurring during the inhibition period showed in some cases complete disappearance of



FIGS. 14–23. Development of unipolar from bipolar forms determined by inhibiting conditions. Figs. 14, 15. Apparent obliteration of both original polarities by inhibiting conditions and determination of new single polarity by the differential of position. Figs. 16–18. Unipolar from bipolar forms by death of one and persistence of other axis. Fig. 19. Equal regression under inhibiting conditions of both hydranths of a bipolar form: the hypostome regions indicated as dotted areas. Fig. 20. Death and disintegration under inhibiting conditions of one axis of a bipolar form with persistence of other. Fig. 21. A form in process of becoming unipolar by death of most of one axis and development of a base from lateral stem region. Figs. 22, 23. Unipolar forms from small fragments of bipolar forms which remained alive while rest was killed by inhibiting conditions.

the more highly specialized structures and the gradual regression of the form to a rounded mass, usually more or less completely inclosed in perisarcal secretion (Fig. 14). After return to water a single apical region developed on the upper surface of this mass and a complete new axis vertical to the substratum resulted (Fig. 15). Such cases were believed to represent the obliteration of the double polarity of the bipolar form and the determination of a new polarity by the differential of position, *i.e.*, the differential between the upper free surface and the surface in contact with

the bottom which supposedly determines a difference in rate of oxygen consumption and discharge of CO_2 and so initiates a new gradient (Child, '26*b*). A case of this sort was described and figured (Child, '15, pp. 144–6, Figs. 75–78; '24, p. 119 and Figs. 106–109). It was also noted in 1910 that some of the bipolar forms became unipolar during the inhibition period. This unipolarity was observed only after the pieces had undergone considerable reduction and was indicated merely by the somewhat elongated form tapering toward one end and an accumulation of perisarc at the slender end (Figs. 16, 17). With long periods of inhibition, *e.g.*, from twelve days to three weeks, such unipolar forms, whether derived from bipolar forms or from the reduction of unipolar forms, might undergo reduction to a length of less than one millimeter and still give rise to normal individuals after return to water (Fig. 18).

At the next opportunity in 1922 to continue these experiments the tank water at the laboratory was apparently somewhat toxic and there was difficulty in keeping material in good condition long enough for such experiments, consequently no further information on these transformations was obtained at that time. In 1924 and 1926 experiments were made with water taken direct from the ocean and it was found that some bipolar forms, more commonly the shorter, underwent regression without actual disintegration of any parts except the two sets of tentacles. Fig. 19 shows a case of this sort in which the two hypostome regions are still distinguishable by their whitish color at the two ends of the piece. They are indicated in the figure as dotted areas. In pieces returned to water at this stage redevelopment of the two hydranths with a new base between them occurred. When the inhibition was continued all indications of the original axes disappeared, although the diameter of the mass might be greater in the direction of the original axes. Such masses may give rise to unipolar forms after return to water and the polarity in such cases appears to be new and determined by the differential of position. But in 1924 and 1926 it was also found by more frequent observation of the material that one axis of a bipolar form is often more susceptible than the other and with a sufficient degree of inhibition it disintegrates, while the other remains alive (Fig. 20). The axis that remains

alive may undergo regression to a condition like Figs. 16 and 17 and in such cases the polarity is one of the two polarities of the bipolar form, or when forms like Figs. 16 and 17 result from the reduction of whole stems or other unipolar forms, it is the original polarity. In such cases the bipolar form becomes unipolar through the death of one axis, rather than by the obliteration of the two original polarities and the determination of a single new polarity. Even in such cases, however, there is much reorganization. The hydranth appearing after return to water is not only absolutely but relatively very much smaller and has a much smaller number of tentacles than the original and the stem and base may develop from regions that were originally part of the hydranth.

The occurrence of this method of change from a bipolar to a unipolar condition is beyond question. It is not properly speaking, a transformation, but merely a persistence of an axis. Since it does occur, the question must be raised whether the apparent transformation by obliteration of the old axes and determination of a single new axis may not actually be merely the persistence of one of the axes. With respect to this point it may be noted, first, that in all cases of disintegration of one axis which have been observed the disintegration occurs early, before the hydranth has lost its form, and second, that both axes have been identified in other pieces after the form of the hydranth was indistinguishable and the only indication of the original axes which was externally visible was the hypostome regions which differed from the rest in color (Fig. 19). No disintegration has been seen in such cases at any later stage and some of them certainly give rise to single new polarities. To all appearances this single polarity is new and determined after obliteration of the old axes in these forms, but in the absence of landmarks after regression of the hydranths it is difficult to be entirely certain as to what happens in cases of this sort. It is hoped that there may be opportunity for further work in the future.

In the light of the data concerning the origin of new polarities in stem pieces (Child, '27*a*, *b*) there is no reason to doubt the possibility of obliteration of old and determination of new polarities in bipolar forms, particularly if some stem is present between the hydranths. Moreover the fusion of hydranths, their reorgani-

zation after regression and the development of basal ends from parts of the hydranth body under experimental conditions indicate that at least the more proximal levels of the hydranth possess considerable capacity for giving rise to other parts, particularly after some degree of regression.

Figs. 14–18 and 20–23 show observed cases of unipolar forms which developed from bipolar forms after subjection to inhibiting conditions. In Figs. 14 and 15 (twelve days in two per cent. alcohol) the single polarity is believed to be new. In Figs. 16, 17 and 18, also twelve days in alcohol, the polarity is almost certainly one of the original polarities, but it has developed proximal and basal regions at the expense of more distal parts. Fig. 19 shows equal regression of both axes, as already noted and Fig. 20 shows a case of disintegration of one axis and persistence of the other. Fig. 21, a bipolar form two days in water after forty hours in alcohol two per cent., is almost completely unipolar, but has a protuberance on one side, which represents the last traces of the other polarity and which later disappears. In this case the basal region has developed from the side of the piece, as in the bipolar-unipolar forms. That is, the unipolar condition has resulted from the death of the more distal regions of one axis and resorption of the remainder and the development of proximal region and base from the side of the stem. The unipolar form in Fig. 22 developed from a bipolar form forty-six hours in alcohol. When returned to water the piece was less than one millimeter in diameter and almost spherical and showed no trace of polarity. Three days later it had attained the stage shown in Fig. 22. Undoubtedly most of the original bipolar form had died, but it was impossible to determine what part of the form the small mass represented, though there is little doubt that it represented the less susceptible proximal region. The persistence of such minute fragments after death of the rest of the piece is of common occurrence under inhibiting condition and in all observed cases, whether they arose from bipolar forms or from stem pieces these masses develop, if they develop at all, as unipolar forms, the polarity apparently being determined by the differential of position. Fig. 23 shows another minute form which developed from a bipolar form after twenty-four hours exposure to NH_4Cl *m*/300 and two

days in water. Here also a large part of the original form disintegrated, but in this case a polarity was evident at the end of the inhibition period, the form of the minute piece being similar to that of Fig. 16. In this case the final polarity undoubtedly represents one of the original polarities of the bipolar form, but completely reorganized.

To sum up: in the development of unipolar from bipolar forms all visible traces of the original polarities may disappear, regression to a rounded mass more or less completely inclosed in perisarc may occur and the axis of the unipolar form appears to be a new gradient determined by the differential of position. In other cases one of the axes of the bipolar form may die and disintegrate under the inhibiting conditions while the other, or some part of it remains alive. In such cases polarity is usually visible, at least in the form of the piece, at all stages and the final single polarity corresponds in direction with this polarity, though it represents extensive reorganization.

Discussion.

The numbers of cases in these transformation experiments are not large. Among forty bipolar forms resulting from reconstitution, subjected to inhibiting conditions sixteen (forty per cent.) transformed into bipolar-unipolar forms, ten (twenty-five per cent.) became unipolar, six (fifteen per cent.) remained bipolar and eight (twenty per cent.) died. This does not include preliminary experiments to determine proper concentrations nor experiments in which all pieces died. Numerous controls in water under good conditions have not shown such transformations in any case. The chief purpose thus far has been to establish the fact of these transformations rather than to determine their frequency. As regards the transformation of bipolar into bipolar-unipolar forms the data are clear and conclusive, but as regards the question of obliteration of both the original polarities and determination of a new single polarity as one method of the origin of unipolar from bipolar forms there is less complete certainty. The course of events in the cases recorded indicates that the two original axes are obliterated unless one resorbs the other after all visible traces of both have disappeared, but if they are so much alike physiologically that they undergo regression equally it is highly improbable that

one is sufficiently different from the other to resorb it. Without resorption or death of one axis, there seems to be no escape from the conclusion that the two original axes are obliterated and a new one determined. That the original single polarity may be obliterated has been shown elsewhere (Child, '27*a*, *b*) and since this is possible there can be little doubt as to the possibility of obliteration of the double polarity of bipolar forms.

The question how the inhibiting conditions transform bipolar into bipolar-unipolar forms is answered very simply from the gradient standpoint. The middle region of the bipolar form where the two polarities meet represents the lowest physiological level present. The inhibiting conditions lower all levels to some extent and may bring the middle region to the level at which a basal region arises. It has already been noted that pieces from the naked stem region commonly secrete more or less perisarc under inhibiting conditions and may become completely inclosed in it (Child, '27*a*). Under normal conditions bipolar forms show almost continuous motor activity, elongation and contraction of the stem and movements of hydranth body and tentacles, consequently no one region is continuously in contact with the bottom. During the inhibition period, however, and for the first day or two after return to water there is little or no motor activity and one side of the piece may be continuously in contact with the bottom. The effectiveness of contact and nearness to the bottom in determining basal ends has been shown in many other experiments (Child, '26*b*, '27*a*, *b*). In the cases under consideration the inhibiting effect of contact and that of the inhibiting agent are additive and the position of the new basal region one one side of the stem may be a resultant of the two factors or the effect of either alone. The important point is that inhibition of the middle region of the bipolar form, whether by chemical agents or by contact with the bottom or by the combined action of both may determine a basal region there.

Torrey ('10) found that the development of a basal region from a lateral stem region might be determined by the more distal levels of the axes already present and without the action of external factors, in other words, under the dominance of the higher levels of the gradients. In the experiments with inhibiting agents, how-

ever, the dominance of the more distal levels is undoubtedly decreased, consequently it cannot be a significant factor in determining the basal region in these cases. This dominance does serve to determine the middle region as the lowest physiological level of the bipolar form, but the inhibiting action of the agent or of contact or of the two combined is required to lower it still further to the level at which the development of a basal end becomes possible.

The more proximal levels and the basal end in these bipolar-unipolar transformations usually attain finally a relatively larger size than under normal conditions. Under all inhibiting conditions used a similar change in proportion from the normal has been observed. This is a result of the differential susceptibility at different levels of the axis. The most active apical regions are more inhibited than the less active proximal and basal regions, consequently the latter are able to obtain a proportionally larger share of the available nutrition or even to grow at the expense of more distal levels and so to become relatively larger than in the normal animals.

The probable transformation of bipolar into unipolar forms by obliteration of the two original polarities and determination of a single new polarity is also easily accounted for in terms of the gradient. Obliteration of the original polarities by the inhibiting agents is a consequence of differential susceptibility of different levels of the gradient. The higher levels being more susceptible are more inhibited than the lower and with certain degrees of inhibition the normal differences of different levels may be greatly decreased or completely obliterated, so far as any appreciable effect is concerned. In the cases of transformation this obliteration is accompanied by regression, involving complete disappearance of the more specialized parts. Under these conditions the differential of position may be effective in determining a new polarity, the upper, freely exposed portion becoming apical, the region in contact or near the bottom basal, as in other cases of obliteration of old and determination of a new polarity by the differential exposure (Child, '27*a*).

The development of unipolar from bipolar forms by the death of one axis and persistence of the other is not, strictly speaking,

an axial transformation, but merely persistence of an axis. The inhibiting conditions kill one axis and not the other because one is more susceptible than the other. In cases of this sort in which it has been possible to distinguish distal and proximal axes of a bipolar form, the distal axis has been found the more susceptible, as might be expected from its development at a higher level of the original gradient of the stem than the proximal. This difference in physiological condition of the two axes is of interest as indicating that even though one of them, the proximal, represents a reversal of the original polarity it still retains traces of its origin from a lower gradient level than the distal axis. Such difference in susceptibility of the two axes is usually found in the longer bipolar forms in which the difference in level of stem at which the two polarities develop is considerable. In the shorter forms with little or no stem between the two hydranths (Fig. 3) there may be no appreciable difference in susceptibility between the two hydranths and both axes may undergo regression equally (Fig. 19).

Persistence of one of the two axes evidently means that some difference in condition persists in the original direction of the gradient. The reorganization which usually occurs with development of a hydranth much smaller than the original and of a basal region consists in development of stem and basal end from what was in some cases originally the proximal part of the hydranth. Apparently the higher levels of the gradient are lowered by the inhibiting conditions more than the lower levels so that apical regions are relatively smaller, proximal and basal regions relatively larger than under normal conditions. This again is a consequence of the differential susceptibility of the different levels of the gradient.

The interpretation of the experimental data in terms of gradients is simple and is in complete agreement with the results of various other lines of experimentation both on *Corymorpha* and on other forms. The existence of the gradients has been demonstrated by many different methods and the differential susceptibility of different levels has been proved beyond doubt, both by differences in survival time and by differential modification of development with many different agents. No other interpretation has as broad an experimental basis as this.

Summary.

1. Bipolar forms resulting from reconstitution of stem pieces of *Corymorpha palma* can be transformed into bipolar-unipolar forms by subjection to inhibiting conditions for various lengths of time. The transformation consists of development of a basal end from the lateral stem region between the hydranths.

2. The development of the base may result from the action of the inhibiting agent in lowering the physiological level of the region concerned to the level at which base development is initiated and the more intimate and continuous contact with the bottom in consequence of decreased motor activity under inhibiting conditions may act in the same direction and contribute to the result.

3. Bipolar forms may become unipolar by the death of the parts representing one axis because of higher susceptibility. In such cases extensive reorganization along the persisting axis occurs.

4. It is believed that inhibiting conditions may bring about transformation of bipolar into unipolar forms by obliteration of the two original polarities through differential susceptibility and the determination of a single new polarity by the differential of position.

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